AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0019] with the following paragraph which has been marked up to show the changes made:

[0019] In another preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said hypoxia-inducible factor (HIF) protein, is the hypoxia-inducible factor 3 alpha protein, also termed HIF3 .alpha., HIF-3 alpha, HIF3 alpha, or HIF3a. The protein HIF3a is also referred to as the HIF3a splice variant 1 (sv1) protein, represented by SEQ ID NO. 2 (FIG. 8) and by the coding sequence of HIF3a sv1(SEQ ID NO. ~~10~~ 28, FIG. 16), and also referred to the HIF3a protein HIF3a splice variant 2 (sv2), represented by SEQ ID NO. 3 (FIG. 9) and by the coding sequence of HIF3a sv2 (SEQ ID NO. ~~11~~ 29, FIG. 17), and also referred to the HIF3a protein HIF3a splice variant 3 (sv3), represented by SEQ ID NO. 4 (FIG. 10) and by the coding sequence of HIF3a sv3 (SEQ ID NO. ~~12~~ 30, FIG. 18), and also referred to the HIF3a protein HIF3a splice variant 5 (sv5), represented by SEQ ID NO. 5, which is similar to protein BAB13865.1 of the Genbank data base (FIG. 11) and by the coding sequence of HIF3a sv5 (SEQ ID NO. ~~13~~ 31, FIG. 19). In the instant invention, said sequences are "isolated" as the term is employed herein. Further, in the instant invention, said HIF3a proteins encoded by the HIF3a gene, HIF3a sv1, HIF3a sv2, HIF3a sv3, HIF3a sv5, are also generally referred to as the HIF3a proteins, or simply HIF3a.

Please replace paragraphs [0047]-[0048] with the following paragraphs which have been marked up to show the changes made:

[0047] In another aspect, the present invention features the use of non-native nucleic acid molecules and/or of translation products, protein molecules of the gene coding for human and/or mouse HIF3a and/or fragments, or derivatives, or variants thereof, of nucleic acid molecules as shown in SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. ~~10~~ 28, SEQ ID NO. ~~11~~ 29, SEQ ID NO.~~12~~ 30, SEQ ID NO. ~~13~~ 31, and protein molecules as shown in SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, as targeting molecules to generate recombinant, genetically altered non-human animals which are transgenic animals and/or knockout

animals. It is preferred that said genetically altered non-human animal is a mammal, preferably a rodent, more preferably a mouse or a rat or a guinea pig. It is further preferred that said genetically altered non-human animal is an invertebrate animal, preferably an insect, more preferably a fly such as the fly Drosophila melanogaster. Further, said genetically altered non-human animal may be a domestic animal, or a non-human primate. In one embodiment, the expression of said genetic alteration results in a non-human animal exhibiting a predisposition to developing symptoms and/or displaying symptoms of neuropathology similar to a neurodegenerative disease, in particular symptoms of a neuropathology similar to AD (AD-type neuropathology), including, inter alia , histological features of AD and behavioural changes characteristic of AD. In another embodiment, the expression of said genetic alteration results in a non-human animal which has a reduced risk of developing symptoms similar to a neurodegenerative disease, in particular a reduced risk of developing symptoms of a neuropathology similar to AD and/or which shows a reduction of AD symptoms and/or which has no AD symptoms due to a beneficial effect caused by the expression of the gene used to genetically alter said non-human animal.

[0048] In one aspect, the invention features a recombinant, genetically altered non-human animal comprising a non-native gene sequence coding for HIF3a, or a fragment or a derivative, or a variant thereof, as shown in SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. ~~10~~ 28, SEQ ID NO. ~~11~~ 29, SEQ ID NO.~~12~~ 30, SEQ ID NO. ~~13~~ 31 and as shown in SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5. Said non-native gene sequence coding for HIF3a may be either the human and/or the mouse HIF3a gene sequence. The generation of said recombinant, genetically altered non-human animal comprises (i) the use of non-native nucleic acid molecules and of translation products, protein molecules of the gene coding for human and/or mouse HIF3a and/or fragments, or derivatives, or variants thereof, as shown in SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. ~~10~~ 28, SEQ ID NO. ~~11~~ 29, SEQ ID NO.~~12~~ 30, SEQ ID NO. ~~13~~ 31 and as shown in SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, for generating a gene targeting construct and (ii) providing said gene targeting construct containing a gene sequence of human and/or mouse HIF3a, or a fragment, or a variant of said gene sequence, and a selectable marker sequence, and (iii) introducing said targeting construct into a

stem cell, into an embryonal stem (ES) cell of a non-human animal, and (iv) introducing said non-human animal stem cell into a non-human embryo, and (v) transplanting said embryo into a pseudopregnant non-human animal, and (vi) allowing said embryo to develop to term, and (vii) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in one or both alleles, and (viii) breeding the genetically altered non-human animal of step (vii) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene. It is preferred that said genetically altered non-human animal expresses a recombinant, an altered gene wherein said expression is a mis-expression, or under-expression, or over-expression, or non-expression. Examples of such targeting constructs containing a gene sequence of human and/or mouse HIF3a and a selectable marker sequence, as well as the expression of said recombinant, altered HIF3a genes in non-human genetically altered animals, preferably animals such as mouse or fly, are disclosed in the present invention (see Example (xii) and FIGS. 39 to 44).

Please replace paragraphs [0088] – [0091] with the following paragraphs which have been marked up to show the changes made:

[0088] FIG. 16 shows the nucleotide sequence of SEQ ID NO. ~~10~~ 28, the coding sequence (cds) of the human HIF3a ~~spliv~~e splice variant 1, comprising 1353 nucleotides (nucleotides 125-1477 of SEQ ID NO. 6).

[0089] FIG. 17 shows the nucleotide sequence of SEQ ID NO.~~11~~ 29, the coding sequence (cds) of the human HIF3a ~~spliv~~e splice variant 2, comprising 1029 nucleotides (nucleotides 23-1051 of SEQ ID NO. 7).

[0090] FIG. 18 shows the nucleotide sequence of SEQ ID NO.~~12~~ 30, the coding sequence (cds) of the human HIF3a ~~spliv~~e splice variant 3, comprising 1899 nucleotides (nucleotides 13-1911 of SEQ ID NO. 8).

[0091] FIG. 19 shows the nucleotide sequence of SEQ ID NO.~~13~~ 31, the coding sequence (cds) of the human HIF3a ~~splive~~ splice variant 5, comprising 1947 nucleotides (nucleotides 226-2172 of SEQ ID NO. 9).

Please replace paragraphs [00143] – [00144] with the following paragraphs which have been marked up to show the changes made:

[0143] First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the HIF3a splice variant 1 encoding gene: 5'-GGGCTCAAGTGATCCTCCTACTT-3' (SEQ ID NO. 10; nucleotides 1466-1488 of SEQ ID NO. 6) and 5'-CATGATGGCACATAGCTGCAGT-3' (SEQ ID NO. 11; nucleotides 1510-1531 of SEQ ID NO. 6) and with specific primers for the HIF3a splice variant 2 encoding gene: 5'-TTTGCGTGAACCTCTGCTTAAG-3' (SEQ ID NO. 12; nucleotides 1305-1326 of SEQ ID NO. 7) and 5'-CACCATGCCAGGCCAAAT-3' (SEQ ID NO. 13; nucleotides 1360-1377 of SEQ ID NO. 7) and with specific primers for the HIF3a splice variant 3 encoding gene: 5'-TCTCTGGCCCTCATTACCTAGCT-3' (SEQ ID NO. 14; nucleotides 1866-1888 of SEQ ID NO. 8) and 5'-CTGTATGACCCTCAACCAGCC-3' (SEQ ID NO. 15; nucleotides 1935-1955 of SEQ ID NO. 8) and with specific primers for the HIF3a splice variant 5 encoding gene: 5'-ACTCTTGGTCTCCCACAGGAAA-3' (SEQ ID NO. 16; nucleotides 2318-2339 of SEQ ID NO. 9) and 5'-AACAGAGCGAGCAGTGCCTT-3' (SEQ ID NO. 17; nucleotides 2380-2399 of SEQ ID NO. 9). PCR amplification (95 °C and 1 sec, 56 °C. and 5 sec, and 72 °C. and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 83.5 °C for the HIF3a splice variant 1 gene specific primers, at 78 °C for the HIF3a splice variant 2 gene specific primers, at 82 °C for the HIF3a splice variant 3 gene specific primers and at about 85 °C for the HIF3a splice variant 5 gene specific primers, with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system

(Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 66 bp for the HIF3a splice variant 1 gene, at 73 bp for the HIF3a splice variant 2 gene, at 90 bp for the HIF3a splice variant 3 gene and at 82 bp for the HIF3a splice variant 5 gene was observed in the electropherogram of the sample.

[0144] In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' (SEQ ID NO. 18) and 5'-AGCCGTTGGTGTCTTTGCC-3' (SEQ ID NO. 19) except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' (SEQ ID NO. 20) and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (SEQ ID NO. 21) (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' (SEQ ID NO. 22) and 5'-GGCAAGGGACTTCCTGTAA-3' (SEQ ID NO. 23). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' (SEQ ID NO. 24) and 5'-GCTAAGCAGTTGGTGGTGCAG-3' (SEQ ID NO. 25). Melting curve analysis revealed a single peak at approximately 83 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' (SEQ ID NO. 26) and 5'-AGCAGTTGGCTGTTGTACCTCTC-3' (SEQ ID NO. 27). Melting curve analysis revealed a single peak at approximately 83 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

Please replace paragraph [0152] with the following paragraph which has been marked up to show the changes made:

[0152] For the immunofluorescence staining of HIF3a protein in cells, a human neuroglioma cell line was used (H4 cells) which stably expresses the human APP695 isoform carrying the Swedish mutation (K670N, M671 L) (H4APPsw cells). The H4APPsw cells were transduced with a pFB-Neo vector (Stratagene, #217561) containing the coding sequence of HIF3a sv3 (HIF3a sv3 cds) (SEQ ID NO. ~~12~~ 30, 1899 bp) and a myc-tag (pFB-Neo-HIF3a sv3 cds-myc, HIF3a sv3-myc vector, 9181 bp, EcoRI/Xhol) under the control of a strong CMV promotor. For the generation of the HIF3a sv3-myc vector, the HIF3a sv3 cds-myc sequence was introduced into the EcoRI/Xhol restriction sites of the multiple cloning site (MCS) of the pFB-Neo vector. For transduction of the H4APPsw cells with the HIF3a sv3-myc vector the retroviral expression system ViraPort from Stratagene was used. The myc-tagged HIF3a sv3 over-expressing cells (H4APPsw-HIF3a sv3-myc) were seeded onto glass cover slips in a 24 well plate (Nunc, Roskilde, Denmark; #143982) at a density of $5x10^4$ cells and incubated at 37 °C at 5% CO_2 over night. To fix the cells onto the cover slip, medium was removed and chilled methanol (-20 °C) was added. After an incubation period of 15 minutes at -20 °C, methanol was removed and the fixed cells were blocked for 1 hour in blocking solution (200 µl PBS/5% BSA/3% goat serum) at room temperature. The first antibody (polyclonal anti-myc antibody, rabbit, 1:5000, MBL) and DAPI (DNA-stain, 0.05 µg/ml, 1:1000) in PBS/1% goat serum was added and incubated for 1 hour at room temperature. After removing the first antibody, the fixed cells were washed 3 times with PBS for 5 minutes. The second antibody (Cy3-conjugated anti-rabbit antibody, 1:1000, Amersham Pharmacia, Germany) was applied in blocking solution and incubated for 1 hour at room temperature. The cells were washed 3 times in PBS for 5 minutes. Coverslips were mounted onto microscope slides using Permafluor (Beckman Coulter) and stored over night at 4 °C to harden the mounting media. Cells were visualized using microscopic dark field epifluorescence and bright field phase contrast illumination conditions (IX81, Olympus Optical). Microscopic images (FIG. 35) were digitally captured with a PCO SensiCam and analysed using the appropriate software (AnalySiS, Olympus Optical).

Please replace paragraph [0154] with the following paragraph which has been marked up to show the changes made:

[0154] Human BACE transgenic flies and human HIF3a transgenic flies were generated according to Greeve et al. (Greeve et al., J. Neurosci. 2004, 24: 3899-3906) and as described in the present invention. A 1942 bp EcoRI/XhoI fragment of the HIF3a sv3 cDNA (SEQ ID NO. 8) containing the entire open reading frame of HIF3a sv3 (SEQ ID NO.~~12~~ 30, SEQ ID NO.4) and fused in frame to a myc-tag (aa EQKLISEEDL) at the 3' end was subcloned into the EcoRI/XhoI restriction sites of the vector pUAST downstream of the GAL4-binding sites UAS (Brand and Perrimon, Development 1993, 118: 401-15). P-element-mediated germline transformation was performed as described by Spradling and Rubin (Rubin and Spradling, Science 1982, 218: 348-53; Spradling and Rubin, Science 1982, 218: 341-7). Twentyeight independent human HIF3a sv3 transgenic fly lines were generated and three different lines were used for the analysis.